Atty Dkt 1[redacted]001
2300-1087
PATENT

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Date

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:

COWGILL et al.

Serial No.: 08/477,984 Group Art Unit: 1654

Filing Date: June 7, 1995 Examiner: A. Gupta

Title: METHODS FOR PURIFYING AUTHENTIC IGF FROM YEAST HOSTS

APPEAL BRIEF TRANSMITTAL

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Further to the Notice of Appeal filed June 4, 1999, transmitted herewith for filing in the above-identified patent application is an Appeal Brief in triplicate. Also enclosed is a Petition for Extension of Time, and a check in the amount of $2,150 to cover the $300 Appeal fee and $1,850 Extension of Time fee.

The Commissioner is hereby authorized to charge any fees under 37 C.F.R. §§ 1.16, 1.17 and 1.21 which may be required by this paper, or to credit any overpayment, to Deposit Account No. 18-1648.

Respectfully submitted,

Date: 1/4/2000

By:
Roberta L. Robins
Registration No. 33,208
Attorney for Applicants

CHIRON CORPORATION
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BRIEF ON APPEAL

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Attorney for Appellants

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BRIEF ON APPEAL

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

INTRODUCTION

Appellants submit in triplicate their brief on appeal in accordance with 37 C.F.R. §1.192. A final rejection was mailed December 4, 1998 (Paper No. 24) in the above-referenced application. In that Action, Examiner A. Gupta finally rejected claims 1, 3-5, 7-12, 17, 18, 47, 49-51, 53-58, 63 and 64 under 35 U.S.C. §103. A response to the final rejection which included a Declaration Pursuant to 37 C.F.R. §1.131, signed by each of the inventors, and a Notice of Appeal were filed June 4, 1999. Attached together with

the required fee is a Petition for Extension of Time of five months, bringing the period for response to January 4, 2000. Appellants respectfully request that the decision of the Examiner be reversed.

I. REAL PARTY IN INTEREST

Chiron Corporation, the assignee of record, is the real party in interest in this matter.

II. RELATED APPEALS AND INTERFERENCES

A Notice of Appeal was filed on June 30, 1999 in commonly owned, related application serial no. 08/990,490, filed December 15, 1997.

III. STATUS OF THE CLAIMS

The application was originally filed with claims 1-24. Claims 25-46 were added in a preliminary amendment dated August 28, 1996. Claims 1-46 were subject to a restriction requirement dated December 23, 1996. Appellants elected to proceed with claims 1-18 and claims 19-46 were subsequently canceled. Claims 47-64 were added in an amendment filed December 10, 1997. Claims 2, 6, 13-16, 48, 52 and 59-62 were canceled in an amendment filed September 16, 1998. Accordingly, claims 1, 3-5, 7-12, 17, 18, 47, 49-51, 53-58, 63 and 64 are pending. These claims have been rejected under 35 U.S.C. §103 and are on appeal.

Prior rejections of various claims under 35 U.S.C. §112, first paragraph; 35 U.S.C. §102(e); and 35 U.S.C. §103 over U.S. Patent No. 5,650,496, to Brierley, in view of Holtz (the particular Holtz reference relied upon was not specified in the Office Action dated March 17, 1998, Paper No. 21) and International Publication No. WO 96/07744 to Bussineau et al. have been withdrawn.

IV. STATUS OF THE AMENDMENTS

A response to the final rejection was filed June 4, 1999. This response included a Declaration Pursuant to 37 C.F.R. §1.131, signed by each of the inventors ("the Rule 131 Declaration"). No claim amendments were made. Despite repeated conversations with representatives of the Patent Office over the past several months, including conversations with Examiner Gupta, Examiner Tsang and Examiner Low, Appellants have been unable to determine the status of the application. Examiner Gupta, in a conversation with the undersigned in November 1999, indicated that the present application might be allowable. However, neither a Notice of Allowance or an Advisory Action have been forthcoming despite subsequent attempts to obtain same. In the absence of input from the Patent Office, Appellants can only guess at the status of the application following the response and Rule 131 Declaration filed June 4, 1999. Based on the conversation with Examiner Gupta in November 1999, Appellants assume that the Rule 131 Declaration has been considered and has been made of record in the application.

V. SUMMARY OF THE INVENTION

The present invention pertains to methods for purifying recombinant, authentic, properly folded insulin-like growth factor (IGF) species which are secreted into a cell culture medium by *Pichia pastoris* cells expressing IGF. As explained at page 2, lines 17-28 of the specification, purification methods for obtaining properly folded IGF from recombinant hosts have been frustrated due to the tertiary structure of the molecule. Particularly, recombinant production of the protein renders a heterogenous mixture which consists largely of inactive, misfolded, insoluble and/or soluble disulfide-linked aggregates. Other aberrant molecules, such as fragments, nicked, oxidized and glycosylated forms, may also be present.

As explained at page 14, lines 20-29 of the application, during fermentation, a variety of IGF forms are secreted into the medium, including analogs, degraded or nicked monomeric forms, oxidized and glycosylated monomers, numerous multimeric

forms, such as dimers, trimers, etc., as well as a major misfolded species which is a disulfide bonded isoform of IGF. Also present is the authentic, properly folded monomeric IGF.

Notwithstanding the presence of these aberrant IGF species, as well as the problems encountered using previous purification methods, the present inventors have discovered a purification technique that provides high yields of authentic, properly folded IGF species. This finding was indeed surprising given the number of failures in the art.

The methods of the invention include a sequential order of steps that yield particular IGF mixtures and, ultimately, a highly purified product. In particular, the methods comprise the following ordered steps: (a) performing cation exchange chromatography; (b) denaturing and renaturing IGF species; (c) performing hydrophobic interaction chromatography; and (d) performing reverse phase high performance liquid chromatography (HPLC). Only a single cation exchange step is used in the claimed methods. See, e.g., page 30, lines 28-29 of the specification.

VI. ISSUES

1. The central issue in this appeal relates to three remaining rejections under 35 U.S.C. §103. The same issue is raised by each of the art rejections:

Whether the Patent and Trademark Office ("PTO") has established a *prima facie* case of obviousness of the claimed invention to a method of isolating an authentic, properly folded IGF polypeptide by the following three combinations despite the fact that Appellants have antedated U.S. Patent No. 5,712,249 to Halloran ("Halloran") which is cited in each of the combinations and despite the fact that Appellants' claims distinguish over the cited combinations. The three art rejections under 35 U.S.C. §103 are as follows:

(i) Claims 1, 5, 7-12, 17, 47, 51, 53-57 and 63 are rejected under 35 U.S.C. §103, as unpatentable over U.S. Patent No. 5,650,496, to Brierley et al. ("Brierley") in view of Halloran;

(ii) Claims 1, 5, 7-12, 17, 47, 51, 53-57 and 63 are rejected under 35 U.S.C. §103, as unpatentable over Halloran in view of Brierley; and

(iii) Claims 1, 3-5, 7-12, 17, 18, 47, 49-51, 53-58, 63 and 64 are rejected under 35 U.S.C. §103 as unpatentable over Brierley in view of Halloran and further in view of International Publication No. WO 96/07744 to Bussineau et al. ("Bussineau") or Halloran in view of Brierley and further in view of Bussineau.

2. An additional issue raised on appeal is the propriety of the objection of claims 47, 49-51, 53-58 and 63 under 37 CFR 1.75 as being substantial duplicates of claims 1, 2-5, 7-12 and 17 despite the fact that no claims have been indicated as allowable by the PTO.

VII. GROUPING OF CLAIMS

Claims 1, 3-5, 7-12, 17, 18, 47, 49-51, 53-58, 63 and 64 are each separately patentable over the cited references. Therefore these claims are divided into 24 separate groups:

(1) claim 1: Independent claim 1 is directed to a method for isolating an authentic, properly folded insulin-like growth factor (IGF) polypeptide from a medium into which the IGF polypeptide has been secreted by *Pichia pastoris* cells expressing the IGF polypeptide. The method involves (a) performing a cation exchange chromatography with the medium to yield a first IGF mixture; (b) denaturing and renaturing IGF species present in the first IGF mixture to yield a second IGF mixture; (c) subjecting the second IGF mixture to hydrophobic interaction chromatography to yield a third IGF mixture; and (d) performing reverse phase high performance liquid chromatography on the third IGF mixture to yield a fourth IGF mixture. The fourth IGF mixture has a greater amount of authentic, properly folded IGF than the first IGF mixture, and, in addition, only one cation exchange step is performed in the method.

(2) claim 3: Claim 3 is directed to the method of claim 1, and further defines that the pH of the medium is raised to between about pH 8 to about pH 12 prior to cation exchange chromatography.

(3) claim 4: Claim 4 is directed to the method of claim 3 and further defines that the pH of the medium is raised to between about pH 10 to about pH 11 prior to cation exchange chromatography.

(4) claim 5: Claim 5 is directed to the method of claim 1 and further defines that the cation exchange chromatography is performed using a sulfopropylated matrix.

(5) claim 7: Claim 7 is directed to the method of claim 1 and further indicates that the denaturing and renaturing steps are performed together using sufficient amounts of a denaturation buffer which includes urea, dithiothreitol, alcohol and salt. The denaturing and renaturing steps are performed under conditions that allow for the reduction and subsequent oxidation of disulfide bonds.

(6) claim 8: Claim 8 is directed to the method of claim 7 and further defines the denaturation buffer as containing about 1 to about 4 M urea, about 1 mM to about 75 mM sodium borate, about .5 M to about 3 M sodium chloride, about 10% to about 30% ethanol and about 0.5- to about 7-fold molar excess of dithiothreitol.

(7) claim 9: Claim 9 is directed to the method of claim 8 and further defines the denaturation buffer as containing about 1.5 M to about 3 M urea, about 3 to about 50 mM sodium borate, about 1 M to about 1.5 M sodium chloride, about 15% to about 25% ethanol, and about an equimolar to about a 5-fold molar excess of dithiothreitol.

(8) claim 10: Claim 10 is directed to the method of claim 1 and further indicates that the hydrophobic interaction chromatography is performed using a polyethyleneamine matrix.

(9) claim 11: Claim 11 is directed to the method of claim 1 and further indicates that the hydrophobic interaction chromatography is performed using a butyl- or phenyl-substituted poly(methacrylate) matrix.

(10) claim 12: Claim 12 is directed to the method of claim 1 and further indicates that the reverse phase high performance liquid chromatography is performed using a C_8 silica-derivatized resin.

(11) claim 17: Claim 17 is directed to the method of claim 1 and further defines IGF as IGF-I.

(12) claim 18: Claim 18 is directed to the method of claim 1 and further defines IGF as IGF-II.

(13) claim 47: Independent claim 47 is directed to a method for isolating an authentic, properly folded insulin-like growth factor (IGF) polypeptide from a medium into which the IGF polypeptide has been secreted by *Pichia pastoris* cells expressing the IGF polypeptide. The method involves (a) performing a cation exchange chromatography with the medium to obtain a partially purified IGF mixture; (b) denaturing and renaturing partially purified IGF species; (c) subjecting renatured IGF species to hydrophobic interaction chromatography; and (d) performing reverse phase high performance liquid chromatography to obtain a further purified IGF mixture. The further purified IGF mixture has a greater amount of authentic, properly folded IGF than the partially purified IGF mixture, and, in addition, only one cation exchange step is performed in the method.

(14) claim 49: Claim 49 is directed to the method of claim 47 and further defines that the pH of the medium is raised to between about pH 8 to about pH 12 prior to cation exchange chromatography.

(15) claim 50: Claim 50 is directed to the method of claim 49, and further defines that the pH of the medium is raised to between about pH 10 to about pH 11 prior to cation exchange chromatography.

(16) claim 51: Claim 51 is directed to the method of claim 47 and further indicates that the cation exchange chromatography is performed using a sulfopropylated matrix.

(17) claim 53: Claim 53 is directed to the method of claim 47 and further indicates that the denaturing and renaturing steps are performed together using sufficient amounts of a denaturation buffer which includes urea, dithiothreitol, alcohol and salt. The denaturing and renaturing steps are performed under conditions that allow for the reduction and subsequent oxidation of disulfide bonds.

(18) claim 54: Claim 54 is directed to the method of claim 53 and further defines the denaturation buffer as containing about 1 to about 4 M urea, about 1 mM to about 75 mM sodium borate, about .5 M to about 3 M sodium chloride, about 10% to about 30% ethanol and about 0.5- to about 7-fold molar excess of dithiothreitol.

(19) claim 55: Claim 55 is directed to the method of claim 54 and further defines the denaturation buffer as containing about 1.5 M to about 3 M urea, about 3 to about 50 mM sodium borate, about 1 M to about 1.5 M sodium chloride, about 15% to about 25% ethanol, and about an equimolar to about a 5-fold molar excess of dithiothreitol.

(20) claim 56: Claim 56 is directed to the method of claim 47 and further indicates that the hydrophobic interaction chromatography is performed using a polyethyleneamine matrix.

(21) claim 57: Claim 57 is directed to the method of claim 8 and further indicates that the hydrophobic interaction chromatography is performed using a butyl- or phenyl-substituted poly(methacrylate) matrix.

(22) claim 58: Claim 58 is directed to the method of claim 47 and further indicates that the reverse phase high performance liquid chromatography is performed using a C_4 to C_{10} silica-derivatized resin.

(23) claim 63: Claim 63 is directed to the method of claim 47 and further defines IGF as IGF-I or an analog thereof.

(24) claim 64: Claim 64 is directed to the method of claim 63 and further defines IGF as IGF-I.

VIII. ARGUMENTS

1. U.S. Patent No. 5,712,249 to Halloran Has Been Antedated Obviating Each and Every Rejection Under 35 U.S.C. §103

The Examiner rejected the claims under 35 U.S.C. §103 over each of the following combinations:

(1) Claims 1, 5, 7-12, 17, 47, 51, 53-57 and 63 stand rejected under 35 U.S.C. §103, as unpatentable over U.S. Patent No. 5,650,496, to Brierley et al. ("Brierley") in view of **Halloran**;

(2) Claims 1, 5, 7-12, 17, 47, 51, 53-57 and 63 stand rejected under 35 U.S.C. §103, as unpatentable over **Halloran** in view of Brierley; and

(3) Claims 1, 3-5, 7-12, 17, 18, 47, 49-51, 53-58, 63 and 64 are rejected under 35 U.S.C. §103 as unpatentable over Brierley in view of **Halloran** and further in view of International Publication No. WO 96/07744 to Bussineau et al. ("Bussineau") or **Halloran** in view of Brierley and further in view of Bussineau.

Appellants note that each of these combinations relies on Halloran (bolded above) as either the primary or secondary reference. Pursuant to 37 C.F.R. § 1.131, Appellants have established that their date of invention is prior to September 8, 1994, Halloran's earliest filing date. See, the Rule 131 Declaration filed June 4, 1999, a copy of which is included in Appendix B. This date is less than one year prior to Appellants' filing date of June 7, 1995. Accordingly, Halloran is not properly citable art and each one of the above bases for rejection is improper.

Specifically, excerpts from an internal production report, attached as an exhibit to the Rule 131 Declaration, establish that Appellants were in possession of the claimed method prior to the earliest filing date of Halloran. In particular, the Rule 131 Declaration establishes that prior to September 8, 1994, a strategy for isolating IGF from yeast cells was developed which, as detailed on page 1 of the exhibit, included a first cation exchange step, a refold step, a hydrophobic interaction chromatography step (HIC), an optional second cation exchange step (see explanation further below) and a reverse phase high performance liquid chromatography (RP-HPLC) step. Page 2 of the exhibit includes the heading "SP-650S" which refers to the second cation exchange step. Page 3 of the exhibit shows that the inventors specifically contemplated the second cation exchange step to be optional if *P. pastoris* was used. Accordingly, there is no longer basis for the remaining rejections and the claims should be allowed.

It is well settled that a section 103 reference may be eliminated by a Rule 131 affidavit containing facts showing completion of the invention commensurate in scope with the claimed invention. *See, e.g.,* MPEP § 715; *In re Asahi/America Inc.,* 33 USPQ2d 1921, 1923 (Fed. Cir. 1995); *In re Wakefield,* 164 USPQ 636 (CCPA 1970). Thus, a Rule 131 affidavit may show prior invention in any one of three ways: (1) reduction to practice of the invention prior to the effective date of the reference; (2) conception of the invention prior to the effective date of the reference coupled with diligence from prior to the reference date to a subsequent (actual) reduction to practice; or (3) conception of the invention prior the effective date of the reference coupled with due diligence from prior to the reference date to the filing of the application (constructive reduction to practice). *See, e.g.,* MPEP § 715.07. Any satisfactory evidence of facts, for

example exhibits of notebook entries, sketches or verbal disclosures can be used to support the affidavit. *See, e.g.,* MPEP § 715.07. Furthermore, the Patent Office is typically required to accept Rule 131 affidavits containing such factual showings at face value, and without investigation. *See, e.g., Herman v. William Brooks Shoe Co.* 39 USPQ2d 1773, 1777 (S.D. N.Y. 1996), citing *Chisum Treatise on Patents*, § 3.08[1][a].

In the pending case, Appellants have submitted a Rule 131 Declaration which includes facts establishing that they were in possession of a method commensurate in scope with the claimed invention prior to the effective date of Halloran. Accordingly, this reference is not available as a reference and the claims are allowable over each of the cited combinations.

2. *Prima facie* Obviousness Has Not Been Established For Any of the Cited Combinations

As explained above, the claims have been rejected as follows:

(1) Claims 1, 5, 7-12, 17, 47, 51, 53-57 and 63 stand rejected under 35 U.S.C. §103, as unpatentable over Brierley in view of Halloran;

(2) Claims 1, 5, 7-12, 17, 47, 51, 53-57 and 63 stand rejected under 35 U.S.C. §103, as unpatentable over Halloran in view of Brierley; and

(3) Claims 1, 3-5, 7-12, 17, 18, 47, 49-51, 53-58, 63 and 64 stand rejected under 35 U.S.C. §103 as unpatentable over Brierley in view of Halloran and further in view of International Publication No. WO 96/07744 to Bussineau et al. ("Bussineau") or Halloran in view of Brierley and further in view of Bussineau.

For the reasons detailed above, the Halloran reference has been removed, thereby obviating these rejections. Even assuming, for the sake of argument only, that Halloran has not been effectively antedated, Appellants submit that there is no teaching, suggestion or motivation within the cited references to support the combinations made by the Examiner. Moreover, no combination of any of these references would result in the claimed methods.

Appellants note that rejections (1) and (2) stated above rely on the exact same references. Similarly, the alternative rejections stated in rejection (3) above also

rely on the same references. Thus, for ease of discussion, Appellants in the following argument will discuss rejections (1) and (2) together and will discuss the alternative rejections in rejection (3) together.

(a) The Examiner has not provided the motivation to combine the references and has relied on prohibited hindsight reconstruction in making the art rejections

The Examiner bears the burden of establishing a *prima facie* case of obviousness. *See, e.g., In re Ryckaert,* 28 USPQ2d 1955, 1956 (Fed. Cir. 1993); and *In re Oetiker,* 24 USPQ2d 1443, 1444 (Fed. Cir. 1992). It is also well established that the Examiner may not combine references to create an obviousness rejection unless there is some suggestion or motivation in the prior art to make the combination. *See, e.g., Arkie Lures, Inc. v. Gene Larew Tackle, Inc.,* 43 USPQ2d 1294 (Fed. Cir. 1997); *In re Oetiker, supra*. Further, even when references relied upon teach that all aspects of the claimed invention are known individually in the art, *prima facie* obviousness is not established without some objective reasoning to combine the teachings of the references. *Ex parte Levengood,* 28 USPQ2d 1300 (BPAI 1993). Even if individual elements of the invention are taught in the prior art, such is not, in and of itself, sufficient to make out a case of *prima facie* obviousness. See, *Symbol Technologies, Inc. v. Opticon, Inc.*, 19 USPQ2d 1241 (Fed. Cir. 1991) ("We do not pick and chose among the individual elements of assorted prior art references to recreate the claimed invention, but rather, we look for some teaching or suggestion in the references to support their use in the particular claimed combination."). As stated by the Court of Appeals for the Federal Circuit, "[i]t is impermissible to use the claimed invention as an instruction manual or 'template' to piece together the teachings of the prior art so that the claimed invention is rendered obvious." *In re Fritch*, 23 USPQ2d 1780, 1784 (Fed. Cir. 1992). See, also, *In re Fine*, 5 USPQ2d 1596, 1600 (Fed. Cir. 1988): "One cannot use hindsight reconstruction to pick and choose among isolated disclosures in the prior art to deprecate the claimed invention." Appellants submit that the cited combinations are indeed based on impermissible

hindsight reconstruction and that a *prima facie* case of obviousness has not been presented by the Office.

> The Examiner has cited Brierley as follows:
>
> Brierley et al. teach a method of purification of IGF that comprises a cation exchange step (sufylpropylated matrix), followed by refolding of the protein with a buffer, followed by hydrophobic-interaction chromatography (butyl substituted polymethacrylate matrix), followed by a second cation exchange step (sufylpropylated matrix) and finally purified by reverse phase chromatography.

Paper No. 24, page 3. The Examiner acknowledges that Brierley fails to teach "deletion of the second cation exchange step" (Paper No. 24, page 3) but alleges that Halloran teaches the use of a single cation exchange step (Paper No. 24, page 3), as well as the use of a reverse phase HPLC using a C_8 matrix (Paper No. 24, page 4). Further, the Examiner recognizes that Halloran "does not teach the denaturing and renaturing step" (Paper No. 24, page 4) but argues that Brierley teaches a purification method that incorporates an "unfolding/refolding step, carried out after the cation exchange step (sulfylpropylated matrix) and before a HIC step..." However, Appellants submit that the Office has failed to establish a *prima facie* case of obviousness based on this combination of references.

In particular, as the Examiner clearly recognizes, the methods recited in independent claims 1 and 47 utilize preparative reverse-phase high performance (high pressure) liquid chromatography (HPLC) following the hydrophobic interaction chromatography step. Brierley, on the hand, does not describe the use of preparative reverse phase HPLC, but rather uses a low performance (low pressure) packing (Amberchrome CG1000sd™) in the reverse phase step. Further, Brierley uses two cation exchange chromatography steps. However, the instant method specifically recites the use of a single cation exchange chromatography step. The Office has therefore cited Halloran to make up for these deficiencies. However, Halloran does not include a denaturation/renaturation step as required by the instant claims. Thus, the Office cites Brierley for providing motivation to include such a step in the claimed methods. It is readily apparent that the Examiner is picking and choosing individual elements from the cited combination and substituting these elements arbitrarily in order to arrive at Appellants' claimed invention. As explained above, this is prohibited.

The combination of references does not teach or suggest a method of isolating an IGF polypeptide from a medium into which the polypeptide has been secreted by *Pichia pastoris* cells expressing the same, wherein the method uses reverse phase HPLC and a single cation exchange column. In fact, Appellants achieve at least comparable, if not better purity, despite the fact that a single cation exchange step is used. See, e.g., Examples IIA and IIB of the application. Appellants submit that a modification of either of Brierley or Halloran, to result in such a method, is not suggested by the cited art. The claimed methods include a number of ordered steps resulting in a highly purified product. These steps may be individually disclosed by the art but are not collectively suggested. The properties of the claimed methods are precisely defined - in the claims themselves, not in the art. To somehow import those properties into hypothetical methods and then to conclude that the hypothetical methods would have exactly the same properties as the claimed methods involves, at the very least, hindsight reconstruction. The Office has picked and chosen among the references based on Appellants' teachings, and relied on prohibited reconstructive hindsight to formulate the present rejection. This it must not do.

Similarly, art rejection (3), set forth above, is also premised on hindsight reconstruction. In particular, this rejection is based on the combination of Brierley in view of Halloran, or Halloran in view of Brierley, and further in view of Bussineau. The Office applies Halloran and Brierley as above. The Office cites Bussineau as follows:

> Bussineau et al. teach a method of recombinant production of IGF where at the end of the fermentation period, an alkaline shock treatment, where an alkali is added to adjust the final pH of the culture medium to the range of 8-11, is conducted...Therefore, it would have been obvious to one of ordinary skill in the art to use use [sic] an alkaline shock treatment, as outlined in Bussineau et al., to obtain a higher yield in protein.

Paper No. 24, page 5. However, Appellants submit that the combination is wholly improper and that this basis for rejection should be withdrawn.

As explained above, the combination of Brierley and Halloran (or Halloran and Brierley) is improper and does not teach or suggest the particular methods of the

invention. Bussineau pertains to methods for improved production of IGF. One method described in Bussineau, "alkaline shock treatment," entails elevating the pH of a fermentor culture at the completion of fermentation and prior to cell removal. See, e.g., page 8, lines 3-5 of Bussineau. Bussineau applies this method to cultures of *Saccharomyces cerevisiae* cells, not *Pichia* cells. At best, then, the addition of Bussineau to the combination of Brierley and Halloran (or Halloran and Brierley) might lead one of skill in the art to incorporate an alkaline shock step into an IGF purification method utilizing *S. cerevisiae* cells, prior to a first cation exchange step. However, the combination certainly does not teach substituting a preparative reverse phase HPLC step for a reverse phase low performance step and does not suggest the invention as a whole. There is no suggestion to modify Brierley's or Halloran's method to that of Appellants' and no indication in Brierley, Halloran or Bussineau that Appellants' methods would be successful for purifying an authentic, properly folded IGF polypeptide. To reiterate, the claims are directed to particular, improved methods having particular elements in particular arrangements. There is, in sum, no motivation provided by the cited references to arrive at the methods as recited in the appealed claims.

2. Additional Arguments Regarding Separately Grouped Claims

Each one of the preceding arguments is applicable to all of the separately grouped claims, i.e., to each claim individually. For the sake of brevity, the arguments have been set our primarily as to independent claims 1 and 47. Claims 3-5, 7-12, 17 and 18 contain all the elements of claim 1. Likewise, claims 49-51, 53-58, 63 and 64 contain all the elements of claim 47. The dependent claims are, therefore, nonobvious for the reasons discussed in detail above. The dependent claims are also further limited in ways that are neither described nor rendered obvious by the cited art.

3. The Office Has Improperly Objected To The Claims Under 37 CFR §1.75

The Office objected to claims 47, 49-51, 53-58 and 63, as being substantial duplicates of claims 1, 2-5, 7-12 and 17. Appellants note that this objection is not proper at this time because no claims have yet been allowed. See, MPEP §706.03(k): "[W]hen

two claims in an application are duplicates, or else are so close in content that they both cover the same thing, despite a slight difference in wording, it is proper after allowing one claim to object to the other claim under 37 CFR 1.75 as being a substantial duplicate of the allowed claim." (Emphasis added.) Furthermore, applicants are given considerable leeway with respect to restating (i.e., by plural claiming) the invention in a reasonable number of ways. See, MPEP §706.03(k). Appellants submit, therefore, that this objection is both improper and premature and should be withdrawn.

CONCLUSION

For the reasons stated above, Appellants respectfully submit that the Office (i) has improperly relied on art that has been properly antedated in making the stated rejections; (ii) even if the reference was not sufficiently antedated, the Office has failed to establish *prima facie* obviousness; and (iii) the objection under 37 CFR §1.75 is improper.

Accordingly, Appellants request that the rejection of the claims on appeal be reversed, and that the application be remanded to the Examiner so that the appealed claims can proceed to allowance.

Respectfully submitted,

Date: 1/4/2000

By: _____
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APPENDIX A (LIST OF CLAIMS ON APPEAL)

Claims (08/477,984)

1. (Amended) A method for isolating an authentic, properly folded insulin-like growth factor (IGF) polypeptide from a medium into which the IGF polypeptide has been secreted by *Pichia pastoris* cells expressing the IGF polypeptide, wherein the method comprises:

(a) performing a cation exchange chromatography with the medium to yield a first IGF mixture;

(b) denaturing and renaturing IGF species present in the first IGF mixture to yield a second IGF mixture;

(c) subjecting the second IGF mixture to hydrophobic interaction chromatography to yield a third IGF mixture; and

(d) performing reverse phase high performance liquid chromatography on the third IGF mixture

to yield a fourth IGF mixture, wherein the fourth IGF mixture has a greater amount of authentic, properly folded IGF than the first IGF mixture, and further wherein only one cation exchange step is performed in the method.

3. The method of claim 1, wherein the method further comprises raising the pH of the yeast cell medium which comprises yeast cells to about pH 8 to about pH 12, prior to the first cation exchange chromatography.

4. The method of claim 3, wherein the method comprises raising the pH of the yeast cell medium which comprises yeast cells to about pH 10 to about pH 11, prior to the first cation exchange chromatography.

5. The method of claim 1, wherein the first cation exchange chromatography is performed using a sulfopropylated matrix.

7. The method of claim 1, wherein the denaturing and renaturing steps are performed together using a denaturation buffer comprising urea, dithiothreitol, alcohol and salt, in sufficient

amounts and under conditions which allow for the reduction and subsequent oxidation of disulfide bonds.

8. The method of claim 7, wherein the denaturation buffer comprises about 1 to about 4 M urea, about 1 mM to about 75 mM sodium borate, about .5 M to about 3 M sodium chloride, about 10% to about 30% ethanol and about 0.5- to about 7-fold molar excess of dithiothreitol.

9. The method of claim 8, wherein the denaturation buffer comprises about 1.5 M to about 3 M urea, about 3 to about 50 mM sodium borate, about 1 M to about 1.5 M sodium chloride, about 15% to about 25% ethanol, and about an equimolar to about a 5-fold molar excess of dithiothreitol.

10. The method of claim 1, wherein the hydrophobic interaction chromatography is performed using a polyethyleneamine matrix.

11. The method of claim 1, wherein the hydrophobic interaction chromatography is performed using a butyl- or phenyl-substituted poly(methacrylate) matrix.

12. The method of claim 1, wherein the reverse phase high performance liquid chromatography is performed using a C_8 silica-derivatized resin.

17. The method of claim 1, wherein the IGF is IGF-I.

18. The method of claim 1, wherein the IGF is IGF-II.

47. (Amended) A method for isolating an authentic, properly folded insulin-like growth factor (IGF) polypeptide from a medium into which the IGF polypeptide has been secreted by *Pichia pastoris* cells expressing the IGF polypeptide, wherein the method comprises:

(a) performing a cation exchange chromatography with the medium to obtain a partially purified IGF mixture;

(b) denaturing and renaturing partially purified IGF species;

(c) subjecting renatured IGF species to hydrophobic interaction chromatography; and

(d) performing reverse phase high performance liquid chromatography to obtain a further purified IGF mixture, wherein the further purified IGF mixture has a greater amount of authentic, properly folded IGF than the partially purified IGF mixture, and further wherein only one cation exchange step is performed in the method.

49. The method of claim 47, wherein the method further comprises raising the pH of the yeast cell medium which comprises yeast cells to about pH 8 to about pH 12, prior to the first cation exchange chromatography.

50. The method of claim 49, wherein the method comprises raising the pH of the yeast cell medium which comprises yeast cells to about pH 10 to about pH 11, prior to the first cation exchange chromatography.

51. The method of claim 47, wherein the first cation exchange chromatography is performed using a sulfopropylated matrix.

53. The method of claim 47, wherein the denaturing and renaturing steps are performed together using a denaturation buffer comprising urea, dithiothreitol, alcohol and salt, in sufficient amounts and under conditions which allow for the reduction and subsequent oxidation of disulfide bonds.

54. The method of claim 53, wherein the denaturation buffer comprises about 1 to about 4 M urea, about 1 mM to about 75 mM sodium borate, about .5 M to about 3 M sodium chloride, about 10% to about 30% ethanol and about 0.5- to about 7-fold molar excess of dithiothreitol.

Claims (08/477,984)

55. The method of claim 54, wherein the denaturation buffer comprises about 1.5 M to about 3 M urea, about 3 to about 50 mM sodium borate, about 1 M to about 1.5 M sodium chloride, about 15% to about 25% ethanol, and about an equimolar to about a 5-fold molar excess of dithiothreitol.

56. The method of claim 47, wherein the hydrophobic interaction chromatography is performed using a polyethyleneamine matrix.

57. The method of claim 8, wherein the hydrophobic interaction chromatography is performed using a butyl- or phenyl-substituted poly(methacrylate) matrix.

58. (Amended) The method of claim 47, wherein the reverse phase high performance liquid chromatography is performed using a C_4 to C_{10} silica-derivatized resin.

63. The method of claim 47, wherein the IGF is IGF-I or an analog thereof.

64. The method of claim 63, wherein the IGF is IGF-I.

APPENDIX B (COPY OF DECLARATION PURSUANT TO 37 CFR §1.131)

Atty Dkt No. 2300-1087

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:

Cowgill et al.

Serial No.: 08/477,984 Group Art Unit: 1654

Filing Date: June 7, 1995 Examiner: A. Gupta

Title: METHODS FOR PURIFYING AUTHENTIC IGF FROM YEAST HOSTS

DECLARATION PURSUANT TO 37 CFR §1.131

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

We, Cynthia Cowgill, Luis Juarbe-Osorio, Patricio Riquelme, Glenn Dorin, Christopher M. Bussineau and Robert D. Kudrna, hereby declare as follows:

1. We are the inventors of the above-captioned patent application ("the Application").

2. We understand that the U.S. Patent and Trademark Office has rejected the claims in the Application in an Office Action mailed December 4, 1998, on the basis of U.S. Patent No. 5,712,249, to Halloran, entitled "Use of Insulin-Like Growth Factors I and II for Inhibition of Inflammatory Response" (the Halloran patent"), which was derived from an application that was first filed on September 8, 1994.

3. We submit this Declaration to show that we had developed a protocol for isolating IGF from yeast, and, in particular, had conceived of a process for isolating IGF from *Pichia pastoris*, as claimed, prior to September 8, 1994. Attached are excerpts from

an internal production report which establish this. The dates and certain information on the submitted pages have been redacted. However, the work described on these pages predates September 8, 1994 and was performed in the United States. Subsequent to the development of the procedure detailed in the appended documents, we worked diligently to further develop and optimize the claimed IGF purification process.

4. In particular, prior to September 8, 1994, we developed a strategy for isolating IGF from yeast cells which, as detailed on page 1 of the exhibit, included a first cation exchange step, a refold step, a hydrophobic interaction chromatography step (HIC), an optional second cation exchange step (see explanation further below) and a reverse phase high performance liquid chromatography (RP-HPLC) step. Page 2 of the exhibit includes the heading "SP-650S" which refers to the second cation exchange step. Page 3 of the exhibit shows that we contemplated the second cation exchange step to be optional if *P. pastoris* was used.

5. We declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Cynthia Cowgill	Date
Luis Juarbe-Osorio	x 02-JUNE 99 Date
Patricio Riquelme	Date
Glenn Dorin	Date
Christopher M. Bussineau	Date
Robert D. Kudrna	Date

REDACTED

Recovery and purification of IGF-1 from recombinant *Saccharomyces cerevisiae* and *Pichia pastoris* cell free supernatants

REDACTED

PROCESS OVERVIEW

1) S-fractogel capture to recover all IGF-1 species, including native, oligomeric, scrambled (misfolded), met-oxidized and glycosylated and eliminate some yeast contaminants.

2) Refolding to generate more native IGF-1 from oligomeric and scrambled species.

3) Concentration/ Diafiltration to eliminate refolding salts and avoid precipitation upon pH drop.

4) PAE (HIC) chromatography to purify native IGF-1, decreasing glycosylated and oligomeric species and substantially reducing yeast contaminants.

5) Diafiltration of PAE pool into SP-650S equilibration buffer to eliminate ammonium sulfate and decrease conductivity.

6) SP-650S chromatography to further decrease the amount of glycosylated species.

7) RP-HPLC (Kromasil) to eliminate met-oxidized, glycosylated and des2-IGF-1 species.

REDACTED

REDACTED

F. SP-650S

REDACTED

REDACTED

*If the *P. pastoris* feedstock is used, this step can probably be eliminated because of the feedstock's higher purity.

G. RP-HPLC

REDACTED

if

Atty Dkt No. 2300-1087

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:

Cowgill et al.

Serial No.: 08/477,984 Group Art Unit: 1654

Filing Date: June 7, 1995 Examiner: A. Gupta

Title: METHODS FOR PURIFYING AUTHENTIC IGF FROM YEAST HOSTS

DECLARATION PURSUANT TO 37 CFR §1.131

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

We, Cynthia Cowgill, Luis Juarbe-Osorio, Patricio Riquelme, Glenn Dorin, Christopher M. Bussineau and Robert D. Kudma, hereby declare as follows:

1. We are the inventors of the above-captioned patent application ("the Application").

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4. In particular, prior to September 8, 1994, we developed a strategy for isolating IGF from yeast cells which, as detailed on page 1 of the exhibit, included a first cation exchange step, a refold step, a hydrophobic interaction chromatography step (HIC), an optional second cation exchange step (see explanation further below) and a reverse phase high performance liquid chromatography (RP-HPLC) step. Page 2 of the exhibit includes the heading "SP-650S" which refers to the second cation exchange step. Page 3 of the exhibit shows that we contemplated the second cation exchange step to be optional if *P. pastoris* was used.

Atty Dkt No. 2900-1087
USSN: 08/477,984
PATENT

5. We declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Cynthia Cowgill — Date: 02 JUNE 99

Luis Juarbe-Osorio — Date

Patricio Riquelme — Date: 4 June 1999

Glenn Dorin — Date: 2 June 1999

Christopher M. Bussineau — Date: 02 JUN 99

Robert D. Kudrna — Date: 02-June-99

REDACTED

Recovery and purification of IGF-1 from recombinant *Saccharomyces cerevisiae* and *Pichia pastoris* cell free supernatants

REDACTED

PROCESS OVERVIEW

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6) SP-650S chromatography to further decrease the amount of glycosylated species.

7) RP-HPLC (Kromasil) to eliminate met-oxidized, glycosylated and des2-IGF-1 species.

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REDACTED

F. SP-650S

REDACTED

REDACTED

*If the *P. pastoris* feedstock is used, this step can probably be eliminated because of the feedstock's higher purity.

G. RP-HPLC

REDACTED

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DATE 1/4/2000

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FOR 08/477,984 2300-1087

Gail Wardwell

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ATTORNEY DOCKET 2300-1087 DATE Jan. 4, 2000
1087.001

IN TRIPLICATE:
Appeal Brief Transmittal; Petition for Extension of Time; Appeal Brief; Appendix A (claims); Appendix B (copy of Rule 131 Declaration); $2150

PAPER: _____

INVENTOR: Cowgill et al.

SERIAL NO.: 08/477,984

FILING DATE: June 7, 1995

RECEIVED BY THE UNITED STATES PATENT AND TRADEMARK OFFICE